REMARKS

The Office Action mailed January 10, 2007 has been carefully considered and the following response prepared. Claims 1-10, 15 and 16 are pending in the application. Claim 1 has been amended as discussed below. Claims 8 and 10 have been canceled without prejudice.

At pages 2 and 3 of the Office Action, the Examiner maintained the rejection of claims 1-10 and rejected claims 15 and 16 under 35 USC 112, first paragraph as failing to comply with the written description requirement. In the present Office Action, the Examiner objected to Applicants' citation to paragraph numbers to show support for specific claim terms because the specification does not contain paragraph numbers.

Applicants again traverse this rejection. In the previous response, Applicants indicated at page 10 that, for convenience, references to the specification refer to the published application. The following response refers to the specification as filed. The Examiner alleged that there is no definition the groups containing "hetero", i.e., heterocyclo and heteroaryls, heterocycloalkyl and alkylheteroaryl groups in the specification. In the claims heterocyclo is not a separate group, but is part of heterocycloalkyl or heterocycloalkanoyl. The definition of heterocycloalkyl and heterocycloalkanoyl can be found in the specification at page 13, lines 1-6 and page 11, lines 18-22. the definition of heteroaryl can be found in the specification at page 13, line 18 to page 14, line 7 (including the table). The definition of alkylheteroaryl can be found in the specification at page 13, line 18 to page 14, line 7 (including the table) and page 11, lines 18-22.

The Examiner further asserted that in various places the claims recite forming a ring with N, O or S, but there is no definition as to which groups are encompassed by this in the specification. Applicants respectfully submit that the specification adequately describes these groups. Substituents R5 and R8, R21 and R22, R24 and R25, and R31 and R32, together with the N, can form a ring with 4, 5, 6, 7, or 8 members, which may optionally contain still another heteroatom selected from the group N, O, and S. The number of carbons in the rings will depend on the identity of the substituents that combine to form the ring and the optional presence of another heteroatom. Persons skilled in the art will be able to determine which nitrogen – containing 4, 5, 6, 7 or 8 member rings can be formed from any two specified substituents.

Lastly, the Examiner asserted that there is no description of the "further agents for tumor treatment" in claim 10 in the specification. Claim 10 has been canceled without prejudice and this rejection is now moot.

In view of the above, withdrawal of this section 112, first paragraph rejection is respectfully requested.

At page 3 of the Office Action, the Examiner maintained the rejection of claims 1-10 and rejected claims 15 and 16 under 35 USC 112, first paragraph as not enabled for the reasons given in the previous Office Action. Briefly, he Examiner indicated that the specification, while being enabled for the compounds, does not reasonably provide enablement for various derivatives of the compounds and method of treatment of tumors, parasites and neurodermitis and for immunosuppression.

Applicants again traverse this rejection. Claim 1 has been amended to delete reference to inclusion complexes. Claim 8, directed to the compounds of claim 1 in the form of an inclusion complexes with cyclodextrin, has been canceled without prejudice. Claim 10 has also been canceled without prejudice.

The type of derivative of the claimed compounds was not specified in the present rejection. Salts are disclosed in the specification at page 14, line 11 to page 15, line 2. Page 15, lines 22-26 and diagram 1 show formation of the tri-potassium salt. Applicants respectfully submit that the specification enables salts of the claimed compounds.

According to the Examiner's comments in the present Office Action, the claims are directed to methods of treatment that are not enabled because of lack of data or guidance showing that the claimed compounds actually treat tumors or parasites. The Examiner's attention is directed to MPEP 2107.03 "Special Considerations for Asserted Therapeutic or Pharmacological Utilities." Applicant does not have to prove that a correlation exists between a particular activity and an asserted therapeutic use as a matter of statistical certainty, nor does the applicant have to provide actual evidence of success in treating humans where such utility is asserted. Instead, all that is required is a reasonable correlation between the activity and the asserted use. Courts have routinely found evidence of structural similarity to a compound known to have a particular therapeutic or pharmacological utility as being supportive of an assertion of

therapeutic utility for a new compound. If reasonably correlated to the particular therapeutic or pharmacological utility, data generated using *in vitro* assays, or from testing in an animal model or a combination thereof almost invariably will be sufficient to establish therapeutic or pharmacological utility for a compound, composition or process.

Applicants submit that the specification enables claims 15 and 16 directed to methods of treating tumors and parasites. At the time the present application was filed, it was known in the art that fredericamycin A and derivatives of fredericamycin A were useful as antitumor and antibacterial substances. The Examiner's attention is directed to U.S. patents 4,584,377 (Yokoi et al.), 4,673,678 (Misra) and 5,166,208 (Kelly et al.) cited in the present Office Action, which each teach the use of fredericamycin A and derivatives as antitumor and/or antibacterial agents. In these patents, the usefulness of the compounds for these purposes was shown in animal models or *in vitro* using different types of tumor cell lines, or different types of microorganisms. None of the patents presents working examples where the compounds were used to treat tumors or infections in patients.

Applicants present the same type of data in the specification at page 57, lines 1-4 in the section entitled "Biological activity against 12 cancer lines" and Table 7. The specification shows the averaged results of the efficacy of over twenty compounds of the invention in *in vitro* assays with twelve cancer cell lines. Adriamycin, cisplatin and fredericamycin, three known antitumor agents were also tested and the results shown in Table 7. In Table 7 the compounds of the invention are shown by a number in the left column that correlates with the example of the same number in the Example section beginning at page 58 of the specification. The claimed compounds showed efficacy in the assays comparable to fredericamycin, and often the IC70 was lower than the IC70 of fredericamycin.

Applicants also submit herewith the publication, U. Abel et al. "Design and semisynthesis of novel fredericamycin A derivatives with an improved antitumor profile," Bioorganic & Medicinal chemistry Letters, vol. 16, pages 3292-3297, 2006, attached as Exhibit A, in support of enablement of the claimed invention. This publication shows antitumor activity of fredericamycin A derivatives in *in vitro* cell assays and a human xenograft model.

The compounds of claims 1-7 are derivatives of fredericamycin, a compound known in the art to have antitumor properties. The specification presents the same type of data as Yokoi et al., Misra and Kelly et al. to show antitumor properties of the compounds. Additionally, antitumor activity of the claimed compounds was compared with the activity of known antitumor agents, fredericamycin and adriamycin in the same assay. Fredericamycin derivatives in Yokoi et al. and Misra had both antitumor and antibacterial activity. Applicants have shown a reasonable correlation between the activity of the compounds and the asserted uses. The data presented in the specification is therefore sufficient to enable to persons skilled in the art to make and use the claimed invention throughout its scope for treatment of tumors and parasites.

The dosage of the compounds of the invention is shown at page 15, lines 5-7. A dose of the compound depends on various factors such as age, condition and weight of the patient, as well as the type of application. The daily dose of active ingredient is usually between 0.1 μg/kg and 1 g/kg orally. With the guidance in the specification persons skilled in the art can arrive at an effective amount of a compound of the invention without undue experimentation.

Withdrawal of this section 112, first paragraph rejection is again respectfully requested.

At page 3 of the Office Action, the Examiner maintained the rejection of claims 1-10, 15 and 16 under 35 USC 103 as being *prima facie* obvious over U.S. patents 4,584,377 (Yokoi et al.); 4,673,678 (Misra) and 5,166,208 (Kelly et al.) and Duan et al., Delgado et al. and Okimoto et al. for the reasons set out in the previous Office Action. Briefly, the Examiner asserted that all of the cited U.S. patents disclose fredericamycin and, with the knowledge of the state of the art at the time of the invention, one skilled in the art would be motivated to make the more soluble and bioavailable salts and sugar residues, and also the cyclodextrin complexes as given in the claims.

Applicants traverse this rejection. Claim 1 has been amended to delete reference to inclusion complexes. Claim 8, directed to the compounds of claim 1 in the form of an inclusion complexes with cyclodextrin, has been canceled without prejudice. Claim 10 has also been canceled without prejudice.

Applicants' prior remarks to this rejection filed October 18, 2006 are incorporated herein by reference.

A *prima facie* case of obviousness requires the following: (1) there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (2) there must be a reasonable expectation of success; and (3) the prior art reference (or references when combined) must teach or suggest all the claim limitations. MPEP at 2143.

The fredericamycin derivatives of claims 1-7, drugs of claim 9 and the methods of claims 15 and 16 are not obvious in view of the combined teachings of Yokoi et al., Misra, Kelly et al. Duan et al., Delgado et al. and Okimoto et al. None of the cited references, alone or in any combination, disclose or suggest the compounds of claims 1-7, the drugs of claim 9 or the methods of claims 15 and 16. The three cited patents, Yokoi et al., Misra and Kelly et al. each disclose fredericamycin A derivatives, but do not disclose or suggest the compounds of claims 1-7. Duan et al., Delgado et al. and Okimoto et al. are concerned with cyclodextrin for use in inclusion complexes with drugs that are poorly water soluble, and there is no disclosure or suggestion in any of these references of the compounds of claims 1-7.

As stated in the specification at page 1, lines 16-21, Applicants surprisingly found that the fredericamycin derivatives of claims 1-7, especially those derivatized in ring A, represent potent drugs. The Applicants' method of introducing groups in ring A semi-synthetically produces compounds having such substitutions in which the water solubility and/or biological effect, in comparison with fredericamycin, can be significantly increased.

The compounds of claims 1-7, drugs of claim 9 and the methods of claims 15 and 16 are not *prima facie* obvious in view of the combined teachings of Yokoi et al., Misra, Kelly et al. Duan et al., Delgado et al. and Okimoto et al.. The combined teachings of the cited references do not disclose or suggest the claimed compounds of claims 1-7, drugs of claim 9 or methods of claims 15 and 16. Withdrawal of this section 103 rejection is respectfully requested.

At page 4 of the Office Action, the Examiner provisionally rejected claims 1-10, 15 and 16 on the grounds of non-statutory obviousness-type double patenting over claims 1-15 of copending application US 2005/0215579 and claims 1-14 of copending application US 2005/0153997.

Applicants traverse this rejection. Applicants submit herewith Terminal Disclaimers for

application US 2005/0215579 (which corresponds to application Serial No.10/520,421) and application US 2005/0153997 (which corresponds to application Serial No.10/511,411). Withdrawal of this double patenting rejection is respectfully requested.

In view of the above, the present application is believed to be in a condition ready for allowance. Reconsideration of the application is respectfully requested and an early Notice of Allowance is earnestly solicited.

Dated: July 10, 2007

Respectfully submitted,

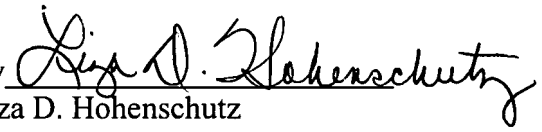
By 

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EXHIBIT A

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Design and semisynthesis of novel fredericamycin A derivatives with an improved antitumor profile

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Abstract—We report the design, semisynthesis, and biological activity of a series of fredericamycin (1) derivatives. Within this series compound **1e** combines low nanomolar cytotoxic potency in vitro, increased tumor cell line selectivity, and in vivo activity in a human xenograft model.

Despite the recent successes of targeted antitumor therapies, the more classic cytotoxic compounds either alone or in combination with other compound classes are still an important element of today's therapies and represent a valuable component of the antitumor product portfolio of modern pharmaceutical industry. The natural product fredericamycin A (**1**, FMA, Fig. 1) is a cytotoxic antitumor lead compound with interesting properties, which never reached clinical development. This was probably due to limited accessibility of sufficient quantities for lead optimization and a general trend away from natural products in the 1990s.

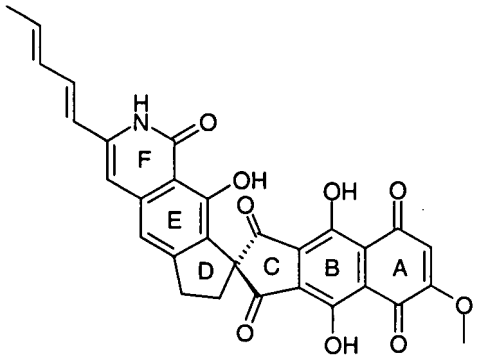

Figure 1. Fredericamycin A (**1**).

FMA is a structurally unique[1] antitumor antibiotic which was isolated from the fermentation broth of a *Streptomyces griseus* strain.[2] It is active in vitro against fungi, gram-positive bacteria, and tumor cell lines, and shows in vivo activity against P388 leukemia, CD8F mammary, and DU-145 prostate tumor xenografts in mice.[3] FMA is a low micromolar inhibitor of both topoisomerases I and II, and inhibits the peptidyl-prolyl *cis–trans* isomerase Pin1 with a K_i of 0.82 μM.[4,3b] The unprecedented architecture along with the biological activity of FMA gave rise to numerous efforts toward the total synthesis of racemic[5] as well as of enantiopure (*S*)-**1**.[6] Nevertheless, there are few reports of synthetic approaches being utilized for the generation of FMA analogues and to establish a structure–activity relationship (SAR). Kelly et al., for example, used their total synthesis approach to produce fredericamycin derivatives exhibiting antiproliferative activity.[7] A series of derivatives was generated starting from a synthetic quinone precursor lacking the F-ring. Introducing various substituted amines into the A-ring yielded compounds with IC_{50}s ranging from 30 to 60 ng/mL in a microdilution assay using six different tumor cell lines. A set of 63 A-ring modified derivatives was produced by reacting synthetic fredericamycin (**1**) with a diverse set of primary and secondary amines. However, no biological activity was given for this series. Kouichi et al. published the acylation of B/E/F-ring hydroxy groups as well as the hydrogenation of the F-ring pentadiene sidechain starting from fermentation derived fredericamycin (**1**) resulting in derivatives with an improved antibacterial activity.[8]

Keywords: Fredericamycin; Antitumor; Spiro quinone; Natural product; Semisynthesis.
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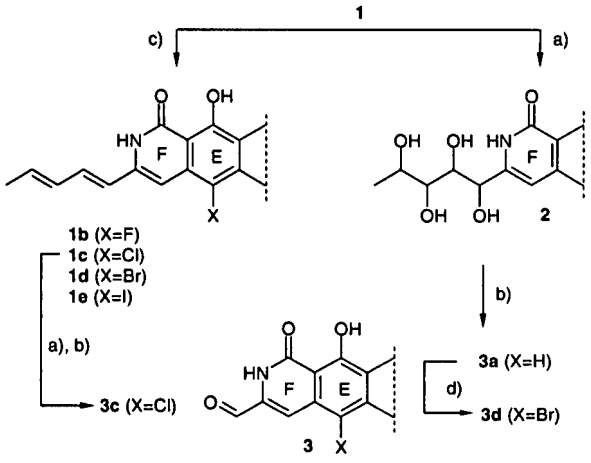
Herein we describe a semisynthetic strategy aiming at FMA derivatives with improved potency and tumor selectivity.[9] Since only limited SAR was available from literature data, the design of derivatives was initially driven by chemical feasibility and for some of the compounds by the overall goal to introduce polar and small pharmacophoric substituents to increase polarity and solubility. Iterative synthetic and testing cycles resulted in the compounds described below.

Multigram quantities of pure **1** could be obtained using an optimized process for the isolation and purification from the fermentation broth of *Streptomyces griseus* strain ATCC49344 mutants without the need for chromatographic purification steps.[10]

Initially, we focused on E- and F-ring modifications of the western portion and envisaged to degrade the F-ring diene sidechain to an aldehyde functionality in order to lower molecular weight and to gain a reactive and versatile functional group for subsequent synthetic transformations. Bis-dihydroxylation of the pentadiene sidechain of **1** resulted in the formation of tetrahydroxylated FMA (**2**) in 87% yield,[11] which was then successfully subjected to diole cleavage using sodium periodate in a mixture of dichloromethane, methanol, and water to generate aldehyde **3a** (Scheme 1). When wet DMSO was used as a solvent for periodate cleavage, we observed the formation of iodinated side-products in LC–MS. This led us to investigate phenolic halogenation using various halogen sources despite being aware that E-ring halogenation would not necessarily increase product polarity and solubility. Conversion of **1** was easily achieved using either Selectfluor® or halosuccinimides yielding E-ring halogenated products **1b** to **1e**.

We attempted to synthesize various E-ring halogenated aldehydes as building blocks for compounds combining E-ring halogenation with replacements of the F-ring pentadiene sidechain. Direct halogenation of fredericamycin-aldehyde **3a** was successful when using bromine in DMF to give bromo-aldehyde product **3d**, whereas treatment of **3a** with *N*-chlorosuccinimide led to decomposition of starting material. Nevertheless, chloro-aldehyde **3c** was finally accessible from chloro-fredericamycin **1c** by bis-dihydroxylation and subsequent diole cleavage. We then started to search for chemoselective conversions of the aldehyde group of halogenated and unhalogenated FMA derivatives leading to derivatives having the FMA pentadiene sidechain replaced by more polar substituents. Treatment of bromo-aldehyde **3d** with a slight excess of various commercially available hydrazines or *O*-alkylhydroxylamines under acidic conditions led to the formation of hydrazones and oximes as exemplified by compounds **4–7** (Scheme 2). Clean conversions were observed in all cases and unreacted reagent and/or starting material could be easily removed using Wang-aldehyde polystyrene and sulfonylhydrazide polystyrene scavenger resin to give products that were typically >95% pure by LC–MS.



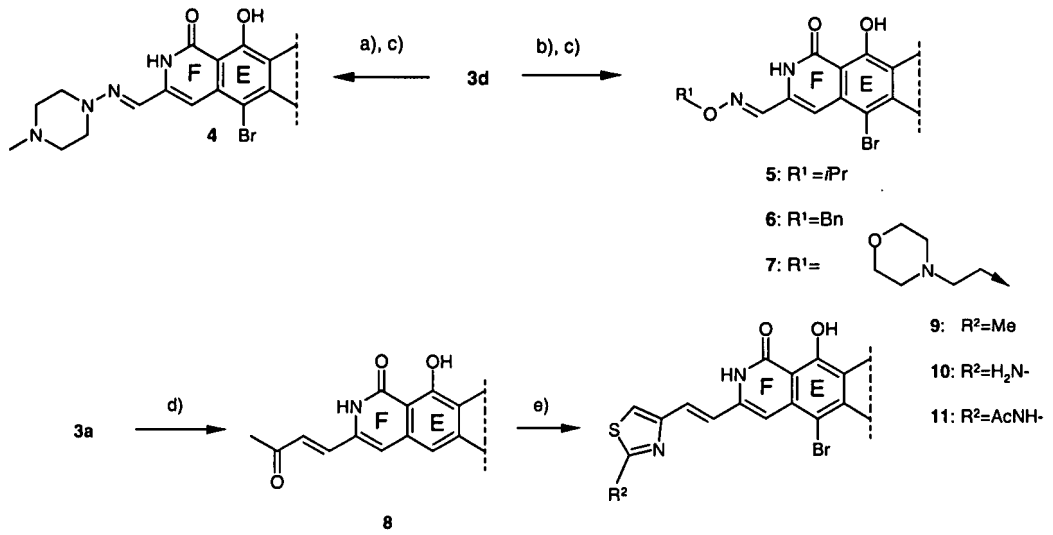
Scheme 1. E- and F-ring modifications. Reagents: (a) NMO, OsO_4, DCM, MeOH, H_2O, 87% from **1**, 75% from **1c**; (b) $NaIO_4$, DMF, H_2O, 100% yield for **3a**, 90% yield for **3c**; (c) for X = F: Selectfluor®, DMF, 14% yield for **1b**; for X = Cl: NCS, DMF, 30% yield for **1c**; for X = Br: NBS, DMF, 32% yield for **1d**; for X = I: NIS, DMF, 17% yield for **1e**; (d) Br_2, DMF, 83% yield.

We then envisaged to introduce F-ring sidechain thiazoles which were thought to be accessible by a short chemoselective synthesis sequence and would eventually undergo additional interactions with the biochemical target. Wittig–Horner olefination toward F-ring butenone **8** was achieved by treating aldehyde **3a** with diethyl 2-oxopropylphosphonate and excess tetramethylguanidine as a base. When **8** was subsequently treated with bromine, bromination of phenolic E-ring as well as of the terminal methyl group occurred. The intermediate α-bromoketone was further converted into thiazole-substituted bromo-fredericamycines **9–11** by reaction with substituted or unsubstituted thioamides.

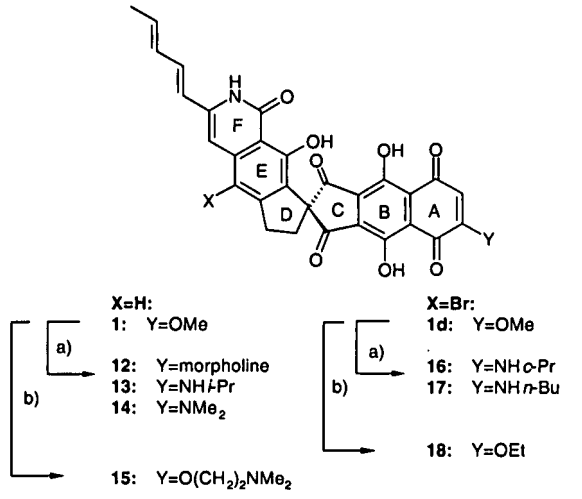
Inspired by Kelly's work,[7] we then started replacing the A-ring methoxy group of FMA (**1**) and the more potent bromo-FMA (**1d**) by nucleophiles such as primary and secondary amines and by alcohols. This might eventually lead to compounds with increased potency and/or an increased stability toward biogenic nucleophiles in vivo. A-ring modifications were possible by exchanging the methoxy group with alcohols (compounds **15** and **18**) or amines (compounds **12–14**, **16**, and **17**) as outlined in Scheme 3. Conversion with amines proceeded smoothly at room temperature in DMF whereas reaction with alcohols required the use of excess reagent, elevated temperatures, and prolonged reaction times.

Attempts to purify synthesis products by either flash chromatography on silica gel or by preparative RP-HPLC resulted in generally low recovery rates. However, we found column chromatography using Sephadex® LH-20 gel (Amersham) and dichloromethane–methanol mixtures as eluent to be the method of choice if purification was required.

Fredericamycin A (**1**) inhibits topoisomerases and Pin1 with low micromolar activity.[4,3b] The published data however do not provide clear evidence that the observed antitumor activity is caused by topoisomerase or Pin1 inhibition. Other targets may contribute



Scheme 2. Sidechain functionalization of aldehydes 3. Reagents: (a) 4-Methylpiperazin-1-amine, TFA, DMF, (b) $RONH_2$, TFA, DMF, (c) polystyrene-CHO, polystyrene-SO_2NNH_2; 90–95% (d) $MeC(O)CH_2P(O)OEt_2$, 1,1,3,3-tetramethylguanidine, 69% yield; (e) 1—Br_2, DMF, 87% yield, 2—$R^2C(S)NH_2$, 62% yield for **9**; 93% yield for **10**; 39% yield for **11**.



Scheme 3. Nucleophilic replacement of A-ring methoxy group. Reagents and conditions: (a) Amine, DMF room temperature; each 99% yield; (b) KOAc, ROH as solvent, 80 °C, 36% yield for **15**, 71% yield for **18**.

to the antitumor effect of fredericamycin A (**1**) and its derivatives. We therefore based the evaluation of the antitumor properties on in vitro cell viability assays using a panel of 10–12 human tumor cell lines and two main parameters: cytotoxic potency and differential cytotoxicity. Potency was expressed as mean of the IC_{70}s of each cell line tested (mean IC_{70}). Differential cytotoxicity was calculated as the fold difference in IC_{70} between the least sensitive and the most sensitive cell line. A selective antitumor compound should display preferential killing of certain cell types. An unspecific membrane disruptor, for example, would kill all cells, whereas a compound targeting a certain tumorigenic pathway would show preferential cytotoxic activity against cell lines, which depend on the respective pathway. A high differential cytotoxicity in a tumor cell line panel is therefore indicative of tumor selectivity and represents an efficient strategy of evaluating the antitumor potential of compound sets in vitro.

Table 1. Cytotoxic activity of FMA derivatives

Compound	Inhibition[a] (%)	Mean IC_{70}[b] (nM)	Differential cytotoxicity[c] (fold)
Paclitaxel	nd[d]	0.7	7847
Camptothecin	nd	8.6	199
Adriamycin	91	39	894
1	82	517	14
1b	91	20	104
1c	91	14	98
1d	92	8.1	100
1e	93	11	837
2	85	186	53
3a	31	nd	nd
3c	25	nd	nd
3d	42	nd	nd
4	73	658	9
5	91	74	47
6	91	50	15
7	88	101	24
8	59	nd	nd
9	85	124	12
10	77	612	7
11	83	512	14
12	98	77	153
13	91	634	21
14	96	306	18
15	94	1019	3
16	97	96	104
17	94	302	20
18	97	160	17

[a] Inhibition: mean inhibition of cell viability of the 10 cell lines at 1 μg/mL.

[b] Mean IC_{70}: cytotoxic potency expressed as mean of the IC_{70}s of the 12 cell lines tested.

[c] Differential cytotoxicity: calculated as the fold difference in IC_{70} between the least sensitive and the most sensitive cell line.

[d] nd, not determined.

Cytotoxic activity[12] of the derivatives was initially evaluated in a panel of 10 tumor cell lines[13] at a fixed compound concentration of 1 μg/mL (Table 1). Compounds exhibiting an inhibition of >70% (mean of the inhibition values of all cell lines) at 1 μg/mL were subjected to dose–response studies using a slightly broader cell line panel[14] and the cytotoxic potency (mean IC_{70}) and differential cytotoxicity values were determined (Table 1).

Halogenation of the E-ring resulted in considerably enhanced potency in the low nM range (**1b**, **1c**, **1d**, and **1e**). Selectivity increased with the size of the substituent (H < F ~ Cl ~ Br < I).

Replacement of the F-ring pentadiene with an aldehyde group resulted in drastically reduced potency (compounds **3a**, **3c**, and **3d**). Other F-ring replacements containing polar groups, such as a hydrazide (**4**) or a carbonyl (**8**) reduced potency as well.

Replacement of the A-ring methoxy group by a morpholine group (**12**) was favorable with respect to selectivity and potency. Other A-ring amines (**13**, **14**, and **15**) showed similar potency and selectivity compared to fredericamycin (**1**). The A-ring vinylogous ester (Michael system) may react covalently with biogenic nucleophiles. Similar or increased potency of the amines (**12–15**) with reduced reactivity of the Michael system compared to **1** suggests that fredericamycin's cytotoxic activity is not the result of such a covalent reaction with a potential target.

Additional work and the identification of molecular targets of fredericamycin (**1**) would be required to decide whether the new functionalities effect potency and selectivity by changes in cellular permeation, stability, and/or interaction with the target.

Figure 2 summarizes the cellular data and compares the cytotoxic potency and the differential cytotoxicity of the novel derivatives with fredericamycin (**1**) and reference compounds. Compound **1e** is outstanding due to its high potency of 11 nM and a differential cytotoxicity of 837-fold compared to fredericamycin (**1**) with 517 nM and 14-fold, respectively. Other compounds showing considerable improvements are the E-ring halogens **1b**, **1c**, and **1d**, the A-ring amines **12** and **16**, and the oxime **5**.

Figure 3 visualizes the cellular activity of fredericamycin (**1**) and two halogenated derivatives (**1d** and **1e**) as mean graphs and demonstrates the improvement in differential cytotoxicity and tumor cell selectivity. Both compounds show pronounced activity against the breast cancer cell line MCF7 and the uterus cancer cell line 1138L. Compounds **1d** and **1e** show similar patterns of cytotoxicity in the cell line panel indicating a similar mode of action.

Based on the available data derivatives **1d** and **1e** are excellent candidates for follow-up testing in in vivo tu-

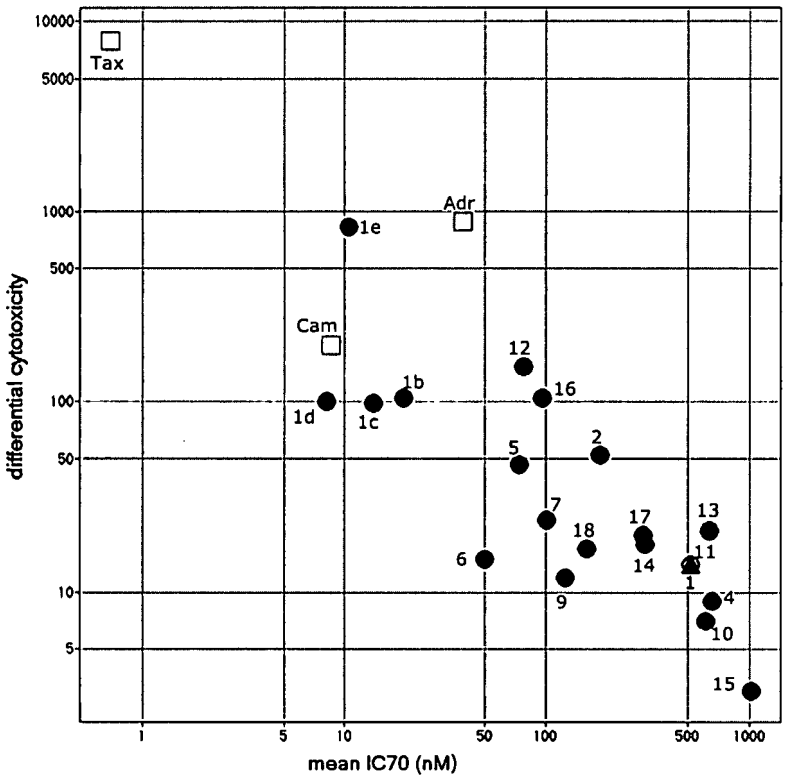


Figure 2. In vitro cytotoxic potency (IC_{70}) and differential cytotoxicity of FMA derivatives. Cell viability and the plotted parameters were determined as described in the text. Mean IC_{70} is plotted against differential cytotoxicity for the lead compound (**1**, black triangle), the reference compounds paclitaxel (Tax, open square), camptothecin (Cam, open square), adriamycin (Adr, open square), and the fredericamycin derivatives (gray circles).

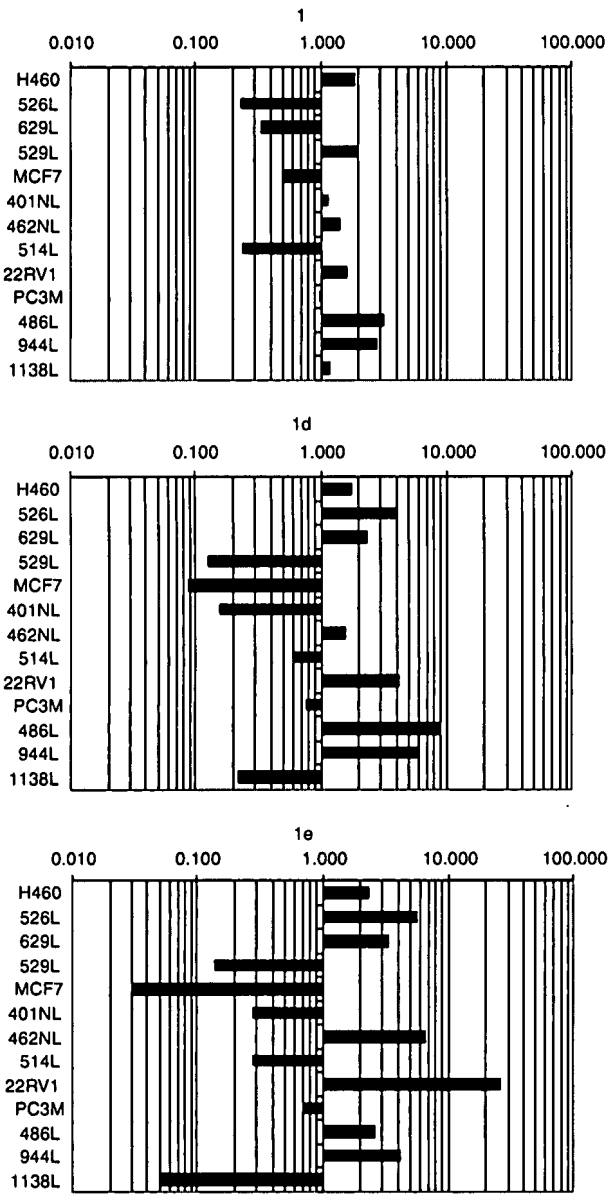


Figure 3. Cellular activity of FMA (**1**) and the halogenated derivatives **1d** and **1e**. Cell viability was determined as described in the text. Variations of individual IC_{70}s from the mean IC_{70} value are plotted as bars on a logarithmic scale. Each bar represents the median of 3–4 (22RV1:2) individual experiments. The mean IC_{70} (517 nM for **1**, 8.1 nM for **1d**, and 11 nM for **1e**) was set to 1. Bars to the left represent lower IC_{70}s, bars to the right higher IC_{70}s compared to the mean. Human tumor cell line panel: non-small cell lung: H460, 526L, 629L, 529L; breast: MCF7, 401NL; melanoma: 462NL, 514L; prostate 22RV1, PC3M; renal: 486L, 944L; uterus: 1138L.

mor xenograft models. At this stage we decided to select compound **1e** for a proof-of-concept experiment in a human xenograft model in nude mice using the human uterine cancer cell line UXF 1138.

The maximum tolerated dose (MTD) was determined in tumor-free nude mice (data not shown). Compound **1e** was toxic at doses of 3 and 1 mg/kg/day. A dose of 0.3 mg/kg/day given on days 0, 4, and 8 resulted in a body weight change of −11% and was considered as the MTD. A dose below the MTD of 0.15 mg/kg/day was chosen for the xenograft experiments. **1e** at 0.15 mg/kg/day given on days 0, 4, and 8 was well tolerated but not effective (data not shown). Given instead on five consecutive days (0–4) compound **1e** produced an antitumor effect with a maximum T/C value (tested group relative to control group) of 51% at day 7 (Fig. 4A). **1e** was well tolerated and resulted only in a slight loss of body weight (−3.1% at day 7, Fig. 4B).

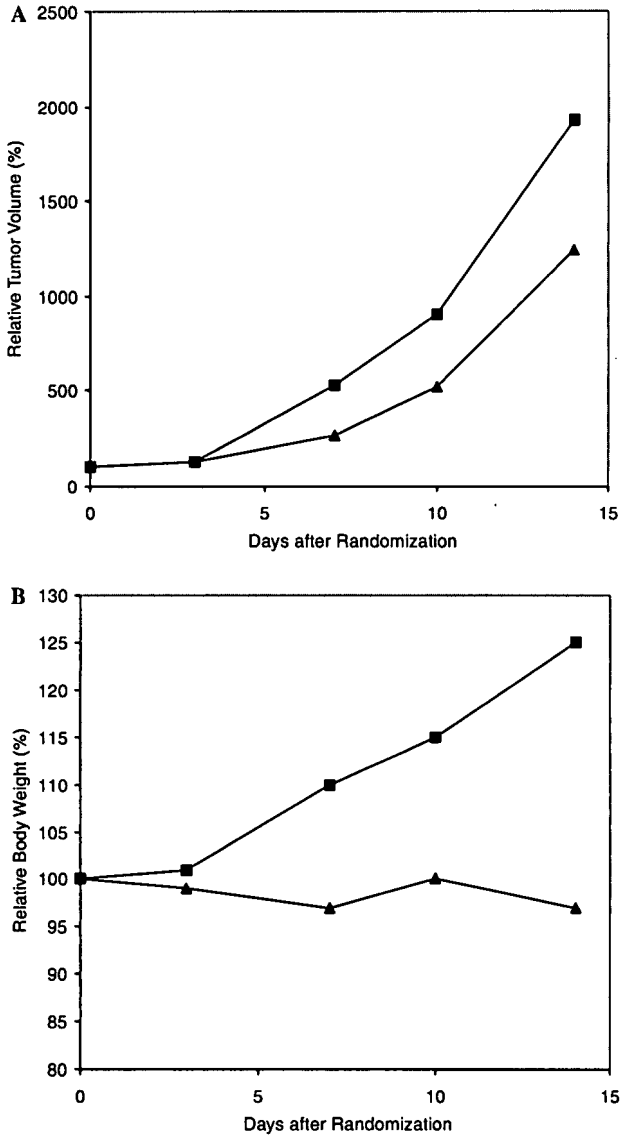


Figure 4. In vivo antitumor activity of derivative **1e** in a human uterine cancer (UXF 1138) xenograft in nude mice. Tumor fragments of about 20 mg were implanted subcutaneously in both flanks of athymic nude mice. Mice were randomly assigned to the treatment and control group with 4–6 mice in each group. The compound was administered ip (3 mL/kg in pure DMSO) at a dose of 0.15 mg/kg/day at days 0–4. Animal weights and tumor diameters were measured twice weekly and tumor volumes calculated. (A) Relative tumor volume, control: black squares, treated: black triangles. (B) Relative body weight, control: black squares, treated: black triangles. Experiments were performed by Oncotest GmbH, Freiburg, Germany.

These first in vivo results are promising but need to be extended to additional FMA derivatives, other tumor cell lines, extended dosing, and optimized administration routes.

In summary, starting from natural fredericamycin (1), a variety of semisynthetic derivatives have been synthesized with modifications of A- and E-rings and of the F-ring pentadiene sidechain. This resulted in compounds with increased, low nanomolar cytotoxic activity in vitro, and increased tumor selectivity. A first set of in vivo experiments revealed promising antitumor activity in an uterine xenograft model.

The results exemplify the power of semisynthetic natural product derivatization for the optimization of natural product drug leads.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.03.029.

References and notes

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11. The relative and absolute stereochemistry of the tetrol sidechain could not clearly be assigned by ^{1}H NMR. However ^{1}H NMR and LC–MS analysis indicate the existence of a single stereoisomer.
12. Human tumor cells were grown at 37 °C in a humidified atmosphere (95% air, 5% CO_2) in RPMI 1640 medium (Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal calf serum (Sigma, Deisenhofen, Germany) and 0.1% gentamicin (Invitrogen). Cells are routinely passaged once or twice weekly, split at a ratio of 1:3–6, and maintained no longer than 20 passages in vitro. A propidium iodide assay was used to assess the effects of the compounds. Cells were harvested from exponential phase cultures by trypsination and plated in 96-well microplates at a density of 5000–12,000 cells/well. After 24 h 20 μL of culture medium (control) or culture medium containing the test compounds was added to the wells. For IC_{70} determination test compounds were applied at 5 concentrations ranging from 0.1 ng/mL to 1 μg/mL. Following 4 days of continuous drug exposure, medium or medium with drug was replaced by 200 μL of an aqueous propidium iodide (PI) solution (7 μg/mL) and the first measurement was performed (excitation 530 nm, emission 620 nm) giving the number of dead cells. The total number of cells is quantified after permeabilizing the cells by freezing/thawing followed by the second measurement. The number of living cells compared to controls is used to calculate % inhibition of cell viability and IC_{70} values.
13. LCL H460 lung, MACL MCF7 mammary, LXFL 529L lung, LXFA 629L lung, MEXF 462NL melanoma, MEXF 514L melanoma, MAXF 401NL breast, RXF 944L renal, RXF 486L renal, and UXF 1138L uterus. The H460 and MCF7 cell lines were from the National Cancer Institute (Bethesda, USA). The other cell lines were established by Oncotest GmbH (Freiburg, Germany).
14. Cell lines as in Reference 13, supplemented by PR PC3M prostate (from the National Cancer Institute) and PRCL 22RV1 prostate (from the American Type Culture Collection, Rockville, MD).